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Short Communication

Interfacial pressure and phospholipid density at emulsion droplet interface using fluorescence microscopy

Jérôme Delacotte, Christine Gourier, Frédéric Pincet*

Laboratoire de Physique Statistique, UMR CNRS 8550 associée aux universités Paris 6 et Paris 7, École Normale Supérieure, 24 rue Lhomond, 75005 Paris, France

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ABSTRACT

Phospholipids are widely used to stabilize oil in water micron size emulsion droplets; the interfacial phospholipid density and tension of such droplets are difficult to estimate. In the present paper, we describe a simple approach by which the measurement of a micron size oil droplet interface fluorescence intensity provides directly both the interfacial phospholipid density and the interfacial tension. This method relies on two prior calibration steps: (i) the quantitative variation of the interfacial tension with fluorescence intensity at droplets interface through micro-manipulation techniques; (ii) the variation of interfacial tension with phospholipid density through monolayer isotherm. Here, we show the validity of this approach with the example of micron size oil droplets stabilized with a phosphatidylcholine phospholipid, in aqueous buffer.

1. Introduction

Characterization of liquid/liquid interfaces is required in emulsion science [1] and in many related industrial processes (e.g. food engineering, cosmetics and pharmaceutics). Moreover, the properties of liquid/liquid interfaces become also predominant when small droplets are manipulated in miniaturized devices [2]. Interfacial tension (IT) of the oil/water interface is a key parameter which can be tuned or modified thanks to surfactant monolayers located at the interface. IT variation is quantitatively related to the surfactant density through a "monolayer isotherm". Several experimental approaches have been used for decades to establish such monolayer isotherms at oil/water interface. The Langmuir trough is usually employed to study oil/water interface and obtain IT and interfacial surfactant densities at the macroscopic scale [3]. This device can also be coupled with fluorescence microscopy [4], in order to investigate phase transitions. Pendant drop techniques based on shape analysis have also been used to perform isotherm measurements on macroscopic (millimeter scale) drops [5,6] at the air/water interface and at the oil/water interface [7]. Small scale systems like emulsion micro-droplets require tools adapted to their size to investigate interfacial properties. Currently, micro-manipulation techniques allow carrying out IT measurements [8] on individual micron size droplets but give no information on the surfactant density. In addition, this measurement relies on deformation of the droplet that is aspirated into a pipette. It can result in a modification or complete destruction of the interface (e.g. in case proteins are present at the interface). To overcome these limitations, we describe here a simple and non-destructive approach by which a simple fluorescence intensity measurement of a micron size oil droplet interface provides simultaneously the interfacial phospholipid density and tension. This method relies on two prior calibration steps: (i) the variation, of IT with fluorescence intensity at droplets interface, F, IT(F), through micro-manipulation techniques; (ii) the variation of IT with phospholipid density, d, IT(d), through monolayer isotherm. The latter is obtained using a setup in which the monolayer at the oil/water interface is formed in a step by step process, increasing the amount of surfactants by successive depositions. Here, we demonstrate on micron size oil droplets in aqueous buffer stabilized by a phospholipid monolayer, that after these two calibration steps, both IT and d can be directly obtained by the simple measurement of F. We chose soybean oil to form droplets and phospholipids as stabilizing surfactants because they are both biological compounds. Soybean oil droplets stabilized by phospholipids are used in food industry and can be used to mimic lipid droplets found in cells of living organisms. In the following, we use "interfacial density" and

* Corresponding author. Tel.: +33 144322502.
E-mail address: pincet@lps.ens.fr (F. Pincet).

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"molecular area" as equivalent information since they are inverse of each other.

2. Methods

2.1. Materials

Phospholipids (1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) (DOPE-RHO)) were purchased from Avanti Polar Lipids in chloroform and used as received. DOPE-RHO was selected as a low bleaching dye carrying the same aliphatic tails as DOPC. The ionic strength and pH of aqueous phase of the emulsion was imposed: the pH of the saline buffer (100 mM potassium chloride (Fluka)) was adjusted at 7.4 using 25 mM Hepes (Sigma Aldrich) and 40% potassium hydroxide solution (Fluka). All solutions were prepared with ultra pure water (Elga). Soybean oil (SO) (CAS 8001-22-7) was purchased from Sigma. It was purified using an activated magnesium silicate (Florisil 100–200 mesh from Sigma) column and stored under inert argon atmosphere at 4 °C. Purification of oil phase (SO and also hydrocarbons) is required before use in studies of oil–water interface (see e.g. [9,10]). SO is mainly composed of mixed triacylglycerides of different chain length (~90% of C_{18} unsaturated and 10% of C_{16} saturated acyl chains) [11]. Contaminant polar components that may compete with phospholipids at the droplet interface were removed from SO [12]. These contaminants may come from the degradation of triglyceride molecules that produce more polar molecules like monoglycerides or fatty acids in addition of contaminants present in the oil. The dynamic IT (IT versus time) was determined before and after purification (see Fig. S1 in ESI). It showed that the IT of unpurified oil decreased on a time scale of 10 s whereas it remained stable (28 ± 0.2 mN/m) in the case of purified oil. Temperature was kept constant during all experiments (21 ± 1 °C).

2.2. Droplets formation

Droplets were formed by shaking oil and buffer for 1 h in 3 mL glass vials. We used VORTEX-GENIE® 2 (Scientific Industries, Inc.) mixer with speed set at 6. Phospholipids (DOPC and PE-RHO) dissolved in chloroform were mixed and added to oil/chloroform (32%, v/v). Volume of chloroform coming with phospholipids can be neglected. The final concentration of phospholipids in emulsion droplets ranged from 75 μM to 375 μM. Buffer was then added to obtain 5% volume fraction of oil–chloroform in water. The droplets size was between 5 μm and 35 μm. With this protocol, the amount of phospholipid embedded in droplets and available for further adsorption at the interface is controlled. Also the presence of chloroform increases droplet density, favoring their sedimention instead of their creaming. This prevents their contamination at buffer/air interface.

2.3. Interfacial tension of droplets

Droplets' interfacial tension was measured using micromanipulation technique. The device was made of a micromanipulator and a pipette holder (Narishige, Japan) under a video microscope. As illustrated in Fig. 1, aspirating a single droplet in a micropipette provided a direct measurement of IT. Each droplet was gently captured with a micropipette through weak aspiration and lifted up in the buffer. Then, the aspiration was slowly increased using a syringe connected to the pipette, causing the oil droplet to enter inside the pipette. Once the oil meniscus into the pipette reached a hemispherical shape, further aspiration increase resulted in a sudden and complete suction of the droplet inside the pipette. The knowledge of this complete aspiration pressure threshold (P_{asp}), the micropipette diameter and the droplet diameter directly provides [8]: $IT = P_{asp}/[4 \times (1/D_p - 1/D_d)]$, where D_p and D_d denote the diameters of the pipette and droplet, respectively.

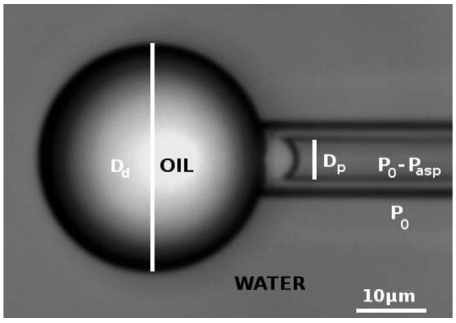

Fig. 1. Micrograph of a micromanipulated oil droplet during IT measurement: the aspiration threshold of the droplet is detected. P_0 is the pressure in the surrounding buffer and IT is expressed as $IT = P_{asp}/[4 \times (1/D_p - 1/D_d)]$.

The sizes of pipette and droplet were obtained by image analysis (ImageJ software provided by NIH). The pressure was measured with a pressure transducer (DP103, Validyne Eng. Corp., USA), the output voltage of which was monitored with a digital voltmeter.

2.4. Fluorescence intensity of droplet interface

The interfaces of droplets were labeled with 2% (w/w) fluorescent phospholipid fraction. Fluorescence images were recorded with a CCD camera (MicroMAX:782YHS), with exposure time of 400 ms. Droplets were shone individually away from other droplets to prevent their photobleaching. Interface fluorescence intensity was measured using ImageJ software. As shown in Fig. 2, a region of interest (ROI) was selected on each droplet. The ROI consisted of a partial circular ring, along the interface, with 260° angular extension and 3 pixels width.

The ROI was centered on the apex of the droplet in order to rule out any artefact due to proximity of the glass pipette. The intensity was averaged in the ROI. We checked that the mean intensity was unsensitive to either small angular or width changes of the ROI.

2.5. Interfacial tension of flat interface

A flat oil/water interface was formed in a glass petri dish (18.8 cm in diameter). The oil upper phase and water lower phase

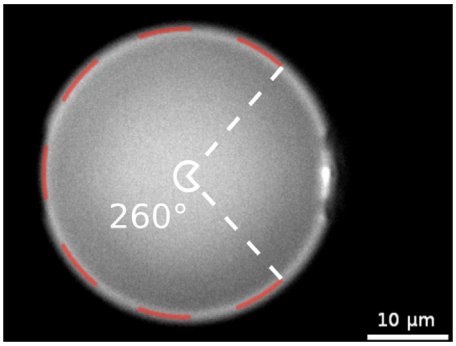

Fig. 2. Micrograph of a micromanipulated droplet, as recorded for interface's fluorescence intensity measurement. The circular red dashed line features the ROI location used to determine *F*, the mean value of interfacial fluorescence intensity. <!–<query>Fig. 2 will appear in black and white in print and in color on the web. Based on this, the respective figure caption has been updated. Please check, and correct if necessary.</query>–>(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)